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Migration of *Caenorhabditis elegans* to manure and manure compost and potential for transport of *Salmonella newport* to fruits and vegetables

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Abstract

A study was done to determine if a free-living, bacterivorous nematode, *Caenorhabditis elegans*, migrates to bovine manure, turkey manure, composted bovine manure, composted turkey manure, and manure-amended soil inoculated with *Salmonella Newport*. Movement of the worm to lettuce, strawberries, and carrots was also studied. *C. elegans* moved most rapidly to turkey manure and strawberries, with 35% and 60% of worms, respectively, associating with samples within 30 min. Survival and reproduction of *C. elegans* in test materials were not affected by the presence of *S. newport*. Bovine manure and bovine manure compost inoculated with *S. newport* (8.6 $\log_{10}$ CFU/g) were separately placed in the bottom of a glass jar and covered with a layer of soil (5 cm) inoculated (50 worms/g) or not inoculated with *C. elegans*. A piece of lettuce, strawberry, or carrot was placed on top of the soil before jars were sealed and held at 20 °C for up to 10 days. In the system using soil inoculated with *C. elegans*, *S. newport* initially in bovine manure was detected on the surface of lettuce, strawberry, and carrot samples within 3, 1, and 1 days, respectively. The pathogen was detected on lettuce, strawberry, and carrot within 1, 7, and 1 days, respectively, when initially present in bovine manure compost. With one exception, the pathogen was not detected on the produce over the 10-day incubation period when *C. elegans* was not present in the soil. Results indicate that *C. elegans* has the potential for transporting *S. newport* in soil to the surface of preharvest fruits and vegetables in contact with soil.

Keywords: *Salmonella newport*; Nematode; *Caenorhabditis elegans*; Manure; Compost; Fruit; Vegetable

1. Introduction

Numerous outbreaks of foodborne illness associated with the consumption of raw or minimally processed fruits and vegetables contaminated with human enteric pathogens have been documented (Beuchat, 2002; IFT/FDA, 2001; NACMCF, 1999). It is often difficult to determine if contamination is a preharvest or postharvest event. These outbreaks have raised interest in identifying processes through which preharvest fruits and vegetables can become contaminated with foodborne pathogens.

Escherichia coli O157:H7, *Listeria monocytogenes* (Anderson et al., 2003; Caldwell et al., 2003b), several serotypes of *Salmonella enterica* (Aballay et al., 2000; Caldwell et al., 2003b; Kenney et al., 2005), *Bacillus cereus* (Anderson et al., 2003), and *Staphylococcus aureus* (Sifri et al., 2003) have been reported to be ingested by *Caenorhabditis elegans*, a free-living, bacterivorous nematode found in soils of temperate regions. The cuticle of live or dead intact worms is thought to provide a physical barrier to protect bacterial cells present in the gut against chemical cleaners and sanitizers applied to processing equipment and some types of raw fruits and vegetables (Caldwell et al., 2003a; Kenney et al., 2004). Chang et al. (1960) reported that chlorinated water is ineffective at killing salmonellae, *Shigella sonnei*, and viruses in the gut of nematodes isolated from water.

It is not uncommon for animal manure and manure compost to be applied to cropland soil as fertilizers. *E. coli*

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O157:H7 can survive in bovine manure-amended soil held at 21 °C for at least 193 days (Jiang et al., 2002). The application of manure and manure compost to soil may attract nematodes that feed on bacteria. Populations of free-living, microbivorous nematodes have been reported to increase in soils to which cattle manure slurry has been applied (Opperman et al., 1993). The extent to which various types of manure and manure composts are incorporated into the soil can influence the population of nematodes. Sand homogeneously amended with a humus–litter mixture has been reported to support higher populations of *C. elegans* compared to sand containing isolated patches of the humus–litter mixture (Mikola and Sulkava, 2001).

It is hypothesized that free-living nematodes such as *C. elegans* and possibly other genera may ingest human pathogens occasionally found in the soil and transport them through the soil matrix. As a worm migrates through soil, it may come in contact with external tissues of plants, either by attraction mechanisms or by random chance. *Salmonella enterica* serotype Poona was detected on cantaloupe rind in contact with soil infested with *C. elegans* within a shorter time compared to rind on soil not containing the nematode (Caldwell et al., 2003b). If infected worms reside on the surface of produce, they may cause contamination by excreting pathogens or as a result of rupturing of the worm cuticle and releasing the gut contents.

Manure and manure composts originating from various animals can have markedly different chemical composition and physical properties. Compared to bovine manure, for example, turkey manure tends to contain more ammonia. Composts generally have lower moisture content than raw manures. The origin and properties of manures and manure composts, both used as fertilizers or amendments to soils in which fruits and vegetables are grown, may affect the attraction, survival, and reproduction of *C. elegans* and other free-living nematodes, and perhaps their potential role as vectors of foodborne pathogens to these commodities.

To date, minimal research has been conducted to determine conditions that may affect the attraction or repulsion of *C. elegans* to fruits and vegetables. Differences in attraction behavior of *C. elegans* may be influenced by the type and amount of volatile chemical compounds released by fruits and vegetables. Various exudates and volatiles released by fruits and vegetables may attract or repel nematodes and other biota in the soil in different ways. Free-living nematodes would be more likely to come in contact with preharvest fruits and vegetable to which they are attracted.

A study was undertaken to determine if *C. elegans* is attracted to bovine manure, turkey manure, composted bovine manure, composted turkey manure, and manure-amended soil inoculated with *Salmonella newport*. Survival and reproduction of *C. elegans* in the same matrices not inoculated with *S. newport* were investigated. Movement of *C. elegans* to lettuce, strawberries, and carrots on an agar medium and the ability of the nematode to transport *S. newport* in soil to the surface of produce was studied.

2. Materials and methods

2.1. Maintenance of C. elegans

A transgenic green fluorescent protein (gfp) labeled strain (PD4792) of *C. elegans* was used. This stain expresses strong fluorescence in the pharynx region. Worms were maintained on K agar (pH 6.5), which contains (per liter of deionized water): potassium chloride (2.36 g), sodium chloride (3.0 g), Bacto peptone (2.5 g; BBL/Difco, Sparks, Maryland), and agar (17.0 g) (Williams and Dusenbery, 1988). *E. coli* OP50, a nonpathogenic strain routinely used as a feed source for *C. elegans*, was grown at 37 °C for 24 h in OP50 broth (5), which contains (per liter of deionized water): sodium chloride (5.0 g) and Bacto peptone (10.0 g). The K agar was surface inoculated with 0.1 ml of a 24-h culture of *E. coli* OP50 and incubated at 37 °C for 24 h to establish confluent growth. Approximately 50 adult worms were deposited on the surface of K agar and incubated at 20 °C for up to 3 days prior to age synchronization of worms.

2.2. Preparation of C. elegans for reproduction and migration experiments

The surface of ten K agar plates, each containing 500–1000 eggs, and 30–50 adult worms, was washed by depositing 5 ml of sterile K medium (Williams and Dusenbery, 1990) and gently rubbing with a sterile bent glass rod. The suspended eggs and worms were aseptically transferred to a sterile 15-ml centrifuge tube. The wash and transfer procedure was repeated to enable efficient harvesting of eggs. Eggs and worms were collected by centrifugation (500×*g*, 2 min) and the supernatant was removed using a pipette. Worms and eggs in a pellet from pooled suspensions were resuspended in 10 ml of 0.013 M NaOH solution containing 1% NaOCl (pH 13.0) and incubated at 20 °C for 15 min to kill all life cycle forms of the worm except the eggs. The suspension was centrifuged (500×*g*, 2 min) and the supernatant was removed. Worms and eggs in the pellet were resuspended in 10 ml of K medium and centrifuged again. The supernatant was removed and the eggs and dead worms were resuspended in K medium. The suspension (0.1 ml containing 400–600 eggs) was deposited on the surface of a K agar plate on which a lawn of *E. coli* OP50 had formed, followed by incubation at 20 °C for 3 days. This procedure ensured that all worms used in assays were of the same age. Adult worms were used in reproduction and migration experiments.

2.3. Bacteria used and preparation of manures and composts for assays

A multidrug resistant strain of *Salmonella newport* was adapted to grow in tryptic soy broth (TSB, pH 7.3; BBL/Difco) supplemented with nalidixic acid (50 μg/ml) (TSBN). Nalidixic acid-adapted *S. newport* was used to facilitate its detection with minimal background interference on tryptic soy agar (TSA; BBL/Difco) supplemented with nalidixic acid (50 μg/ml)

(TSAN). Nalidixic acid-sensitive bacteria naturally occurring in soil, manures, and manure composts and *E. coli* OP50 cells remaining on the surface or in the gut of the worm as a result of feeding would not be expected to grow on TSAN but nalidixic acid-adapted *S. newport* would form colonies. Nalidixic acid-adapted *S. newport* was grown in 10 ml of TSBN at 37 °C for 24 h. The pathogen was transferred twice to 10 ml of TSBN by loop inoculum (ca. 10 μl) at successive 24-h intervals. Cells were collected by centrifugation (2000×*g*, 10 min), resuspended in 10 ml of sterile deionized water, collected again, and resuspended in 10 ml of deionized water. This suspension served as an inoculum for *C. elegans* attraction experiments.

Turkey manure mixed with litter, turkey manure compost, separated bovine solids (bovine manure), and bovine manure compost were supplied by the United States Department of Agriculture, Agricultural Research Service (Beltsville, Maryland). Soil (Redi-Earth Peat-Lite Mix, 3CP, The Scotts Company, Columbus, Ohio) (pH 6.9) was saturated with water and combined with the turkey manure or bovine manure (9:1, w/w). Samples (40 g) of manures, manure composts, and manure-amended soils were placed in individual quart Ziploc® storage bags (S.C. Johnson and Son, Inc., Racine, Wisconsin) and inoculated with 2 ml of a washed cell suspension of *S. newport* to give 8.55 $\log_{10}$ CFU/g. Bags containing inoculated samples were sealed and vigorously shaken for 30 s to uniformly distribute the pathogen. Before using in assays, samples were incubated at 20 °C for 24 h.

Populations of nalidixic acid-adapted *S. newport* in samples incubated at 20 °C for 0, 1, 3, 5, and 7 days were determined. Samples (2.2 g) were individually placed in a stomacher 80 bag (Seward Medical Ltd., London, UK) containing 19.8 ml of sterile 0.1% peptone water. Bags containing slurries were pummeled in a stomacher 80 (Seward Medical) for 60 s at high speed. Undiluted samples (0.25 ml in quadruplicate and 0.1 in duplicate) and serially diluted samples (0.1 ml in duplicate) were spread plated on TSAN and incubated at 37 °C for 24 h. Presumptive *S. newport* colonies were enumerated and random colonies were confirmed using a *Salmonella* latex agglutination assay (Oxoid, Basingstoke, UK).

2.4. Survival and reproduction of C. elegans

Manures, manure composts, and manure-amended soil were prepared as described above. Samples (2.2 g) inoculated with *S. newport*, as well as uninoculated soil, manures, manure composts, and manure-amended soil samples, were individually placed in 35 mm diameter × 10 mm deep Petri dishes. Ten worms suspended in 10 μl of K medium were deposited on top of each sample. The Petri dish was covered, placed in a tub, and sealed by applying a lid. Samples were incubated for 1, 3, 5, and 7 days at 20 °C before analyzing for populations of *C. elegans*. Each sample was placed in a sterile 50-ml centrifuge tube containing 10 ml of Ludox® TM-30 colloidal silica (Sigma-Aldrich, St. Louis, Missouri) and the mixture was centrifuged (500×*g*, 2 min). Supernatant containing worms was decanted into a Petri dish (100 mm diameter × 15 mm deep) and examined with a dissecting microscope to determine if numbers of *C. elegans* declined (−), did not change (nc), or increased (+ [up to 200%] or ++ [>200%]) compared to initial populations. All experiments were replicated three times.

2.5. Migration of C. elegans to manure, manure compost, and manure-amended soil inoculated with S. newport, and to uninoculated soil

Four plastic rings (2 cm diameter, 0.2 cm long) were placed on the surface of TSAN equidistant (ca. 5 cm) from each other around the perimeter of a Petri dish (100 × 15 mm). Samples (ca. 0.5 g) of uninoculated soil and bovine manure, bovine manure compost, and bovine manure-amended soil inoculated with *S. newport* were placed in the four rings; the same procedure was done using uninoculated soil and turkey manure, turkey manure compost, and turkey manure-amended soil inoculated with *S. newport*. Forceps were used to remove the rings, leaving samples on the surface of the agar.

A K agar plate containing a lawn of *E. coli* OP50 on which adult worms had developed was flooded with 10 ml of K medium. The suspension was transferred to a sterile 15-ml tube and centrifuged (500×*g*, 2 min). The supernatant was removed and the pellet was resuspended in 1.0 ml of K medium. The worms were allowed to settle to the bottom of the tube for 5 min at 20 °C. A suspension (10 μl) containing 20–30 worms was deposited onto the surface of the TSAN agar plate 3.5 cm from each of the four sites where test samples had been placed. The number of worms in the inoculum was recorded. The surface tension of the inoculum was carefully broken with a sterile fine-bristle paint brush to facilitate worm contact with the TSAN agar surface. Migration of worms toward the test samples was monitored at 5-min intervals for 30 min. This experiment was replicated five times.

2.6. Migration of C. elegans to lettuce, strawberries, and carrots on TSAN

Lettuce (*Lactuca sativa* L.), strawberries (*Fragaria* × *ananassa* Duchesne), and carrots (*Daucus carota* L.) were purchased from a supermarket in Griffin, Georgia and held at 20 °C until used in experiments the same day. Circular (1.5 cm diameter) pieces were cut from produce with no. 9 cork borer which had been sterilized by immersing in 70% ethanol. The circular piece of produce, skin side down, was placed on the surface of TSAN. Two plastic rings (2 cm diameter, 0.2 cm long) were also placed on the surface of TSAN at positions ca. 5 cm apart to form the points of an equilateral triangle that included the site of the piece of produce. One ring was filled with either *S. newport* inoculated bovine manure or bovine manure compost, both inoculated with *S. newport*, and the other ring was filled with uninoculated soil. The rings were removed from the agar using a pair of forceps and *C. elegans* (20–30 worms) suspended in K medium was placed on the surface of the agar plate ca. 1.8 cm from each test sample. The surface tension of the suspension was broken and attraction of worms to test samples was monitored as described above. This experiment was replicated three times.

2.7. Migration of S. newport in soil to lettuce, strawberries, or carrots

Circular (1.5 cm diameter) pieces were cut from produce with no. 9 cork borer sterilized by immersing in 70% ethanol. Bovine manure (1 g) or bovine manure compost (1 g), both inoculated with *S. newport* as described above, were separately placed into a sterile 20-ml disposable scintillation jar (Fisher Scientific, Pittsburgh, Pennsylvania). Soil (9 g) inoculated with *C. elegans* (ca. 50 worms/g) or not inoculated with the worm was placed on top of the manure or compost inoculated with *S. newport* (5 cm deep). A piece of lettuce, strawberry, or carrot was firmly placed on top of the soil. In another set of jars, bovine manure, bovine manure compost, and soil were prepared as described above, except lettuce, strawberry, and carrot samples were not placed on top of the soil. Jars were covered with parafilm and lids were applied to prevent evaporation of water. Samples were incubated for 1, 3, 5, 7, and 10 days at 20 °C before analyzing produce or the top layer of soil (1 cm deep) for the presence of *S. newport*.

Pieces of lettuce, strawberry, carrot, or ca. 2 g of soil removed from the top 1 cm layer was separately placed into a stomacher 400 bag and 100 ml of lactose broth (pH 6.9, BBL/Difco) supplemented with nalidixic acid (50 μg/ml) (LBN) was added. Mixtures were incubated at 37 °C for 24 h. A loopful of LBN was inoculated into 10 ml of Rappaport–Vassiliadis enrichment broth (RV) (pH 5.2, Oxoid) and incubated at 42 °C for 24 h, followed by streaking onto bismuth sulfite agar (pH 7.7, BBL/Difco) supplemented with nalidixic acid (50 μg/ml) (BSAN) and incubating at 37 °C for 24 h. Randomly selected presumptive-positive colonies of *S. newport* that formed on BSAN were confirmed by *Salmonella* latex agglutination assays. The presence or absence of *S. newport* was recorded as either positive (+) or negative (−), respectively. This experiment was replicated four times.

2.8. Statistical analysis

Each experiment was replicated at least three times. Data were analyzed using Statistical Analysis Software (SAS Institute, Cary, North Carolina). Significant differences ($P \leq 0.05$) between values were determined using *t*-test.

3. Results and discussion

3.1. Survival and reproduction of C. elegans

Populations of *C. elegans* remained unchanged for 1 day at 20 °C in manures, manure composts, manure-amended soil, and soil inoculated or not inoculated with *S. newport*, but increased or decreased, depending on the test material between 1 and 7 days (Table 1). Populations of *C. elegans* were highest in turkey manure and bovine manure incubated for 3–7 days at 20 °C. Manures had the highest moisture contents (62.2–79.8%) of all products examined. Microbial populations and profiles in fresh manure can change rapidly. Manures may contain nutrients that can be readily utilized by bacteria to reproduce and maintain population size, despite predation by *C. elegans*. Younger cells of *S. newport* may have been more attractive to *C. elegans*, resulting in an increase in the rate of reproduction. It has been reported that *C. elegans* is more readily attracted to young (24–48 h) bacterial colonies than to older (96–192 h) colonies on agar media (Grewal and Wright, 1992). Attraction of *C. elegans* to bacteria is also dependent on the bacterial species. *Acinetobacter calcoaceticus* var. *antratus* attracts *C. elegans* within 192 h, but the worm is not attracted to *Serratia liquefaciens* at the same age (Grewal and Wright, 1992). Anderson et al. (2003) also observed different levels of attraction of *C. elegans* to surrogates of enteric pathogens of the same physiological age.

Table 1
Change in the number of *Caenorhabditis elegans* in manure, manure compost, manure-amended soil, and soil inoculated with *Salmonella newport*

Material inoculated with *S. newport*	Change in number of *C. elegans*[a]			
	1	3	5	7 days
Bovine manure	nc	++	nc	nc
Bovine manure compost	nc	−	nc	nc
Bovine manure-amended soil	nc	+	nc	nc
Turkey manure	nc	++	nc	nc
Turkey manure compost	nc	−	nc	nc
Turkey manure-amended soil	nc	+	+	nc
Soil	nc	−	nc	nc

[a] Change, over sampling days, in number of *C. elegans* in manure, manure compost, manure-amended soil, and soil on day 1 relative to the initial number (ten) in inoculated samples (day 0) or on days 3, 5, and 7 relative to days 1, 3, and 5, respectively. Changes in number of *C. elegans* during incubation at 20 °C for up to 7 days were recorded as − (decrease), nc (no change), + (up to 200-fold), or ++ (>200-fold).

Populations of *C. elegans* began to decline in turkey manure compost, bovine manure compost, and soil between 1 and 3 days at 20 °C (Table 1). Few (<10 viable worms/sample) or no juvenile worms were observed in composts at subsequent sampling times. The moisture content of the turkey manure compost (31.5–36.2%) and bovine manure compost (59.6–61.9%) may have been insufficient to enable survival or support reproduction of *C. elegans.* The presence of *S. newport*, together with bacteria naturally occurring in composts, would probably have been sufficient to support reproduction of *C. elegans*. Soil to which compost has been added for the purpose of growing fruits and vegetables would likely have higher moisture content than the compost tested in this study. Periodic rainfall or irrigation increases the moisture content of soils and can enhance bacterial survival and growth. Gagliardi and Karns (2000) conducted a study in which water was applied at a rate of 1.65 cm/h to sandy loam and silty clay loam soils inoculated with *E. coli* O157:H7. Hourly analysis of leachate samples revealed that numbers of *E. coli* O157:H7 remained near the inoculum level for at least 8 h. This indicates that the pathogen was multiplying to maintain high numbers in the leachate. The moist environment of soil and compost-amended soil would promote the growth of bacteria to be used as nutrient sources by *C. elegans*.

C. elegans increased from an initial population of 10 worms/2.2-g sample in manure-amended soil to ca. 20 worms/2.2-g sample between 1 and 3 days and ca. 50 worms/2.2-g

sample between 3 and 5 days, but remained constant between 5 and 7 days. The increase between 1 and 3 days was not as large as that in manures. The worms may have been more closely associated with the manure solids and to nutrients in the manure than to soil particles in the manure-amended soil. Results are in agreement with those reported by Mikola and Sulkava (2001) showing that *C. elegans* populations were higher in humus–litter than in sand in a heterogeneous mixture.

Little is known about the general attraction characteristics of free-living nematodes to enteric pathogens in soil amended with manure or manure compost. *C. elegans* is not commonly studied outside of the laboratory environment, possibly because it lacks economic impact on agricultural crops. *Diploscapter* sp., in contrast, is reportedly found in a range of agricultural habitats (Siddiqi, 1998) and has a higher thermal tolerance compared to *C. elegans* (Lemzina and Gagarin, 1994). Lawns of *S.* Poona, *E. coli* O157:H7, and *L. monocytogenes* have been shown to support the survival and reproduction of *Diploscapter* on agar media and in manures (Gibbs et al., in press). The worm was also able to disperse pathogenic bacteria after it was exposed to the pathogens in soil.

3.2. Migration of *C. elegans* to manure, manure compost, and manure-amended soil inoculated with *S. newport* and to uninoculated soil

The mean number of worms that migrated to test materials inoculated with *S. newport* or to uninoculated soil after 5, 10, 15, 20, 25, and 30 min at 20 °C was used to calculate changes in the percent distribution of *C. elegans.* Attraction of *C. elegans* to test samples is shown in Fig. 1. Regardless of the origin of manures and composts, *C. elegans* was associated with these materials in greater numbers compared to uninoculated soil. The largest percentage of *C. elegans* was observed in turkey manure. In general, worms that entered samples of turkey manure or turkey manure-amended soil remained associated with these matrices for the duration of the 30-min incubation time. At the end of the 30-min test period, 45.4% of the worms initially deposited on the plates were detected in turkey manure.

Turkey manure had the strongest odor of materials tested and it is possible that *C. elegans* is attracted to one or more of the aromatics, e.g., ammonia, released by the manure. Turkey manure, turkey manure compost, bovine manure, and bovine manure compost had ammonia concentrations of 5.39, 0.01, 1.45, and 0.89 mg/kg dry weight, respectively. Fewer numbers of *C. elegans* were observed in soil amended with turkey manure than in turkey manure. Within 5 min of depositing worms on the agar surface, 7–8% of worms on agar had entered turkey manure-amended soil compared to 23–37% of worms in manure.

Compared to turkey manure and turkey manure compost, *C. elegans* was much less often associated with bovine manure and bovine manure compost. Fewer than 10% of the worms were associated at any incubation time. Higher numbers of *C. elegans* were located in or near bovine

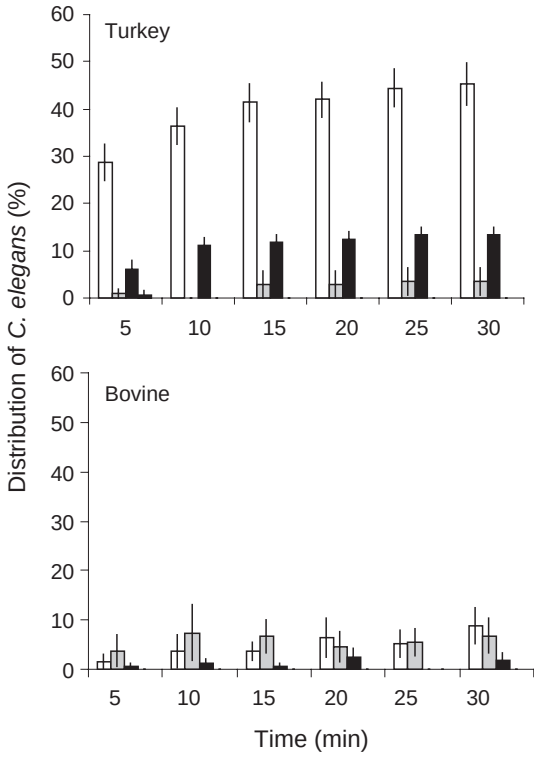

Fig. 1. Migration of *C. elegans* on TSAN on which turkey or bovine manure, manure compost, and manure-amended soil inoculated with *S. newport* and uninoculated soil were deposited. Worms (20–30) were deposited 2.5 cm away from samples. The percentages of worms distributed in manure (open bars), manure compost (shaded bars), in manure amended soil (solid bars), or uninoculated soil (hatched bars) within 30 min at 20 °C were monitored. Some of the worms did not migrate to manure, manure compost, or soil.

compost than other test materials between 5 and 10 min, but equal or higher numbers of *C. elegans* were observed in bovine manure after 10 min. A maximum of 7% of the worms were associated with the soil amended with bovine manure over the 30-min test period.

3.3. Migration of *C. elegans* to lettuce, strawberries, and carrots

The mean number of worms that migrated to lettuce, strawberries, or carrot, bovine manure or bovine manure compost inoculated with *S. newport*, or uninoculated soil after 5, 10, 15, 20, 25, and 30 min at 20 °C was used to calculate the percent distribution of *C. elegans*. The percent distribution of *C. elegans* in produce, bovine manure, and soil is shown in Fig. 2. *C. elegans* migrated in higher numbers to produce and manure inoculated with *S. newport* compared to uninoculated soil. *C. elegans* was most strongly attracted to strawberries in preference to inoculated bovine manure or uninoculated soil. The number of *C. elegans* associated with the strawberry increased over the 30-min sampling time. *C. elegans* was less likely to migrate to lettuce and carrot than to strawberry. No more than 10% of worms were associated with lettuce at any given time during the 30-min test period. The number of *C. elegans* in manure increased slightly between 10 and 30 min. At 25 and 30 min, the percentage of *C. elegans* in manure was

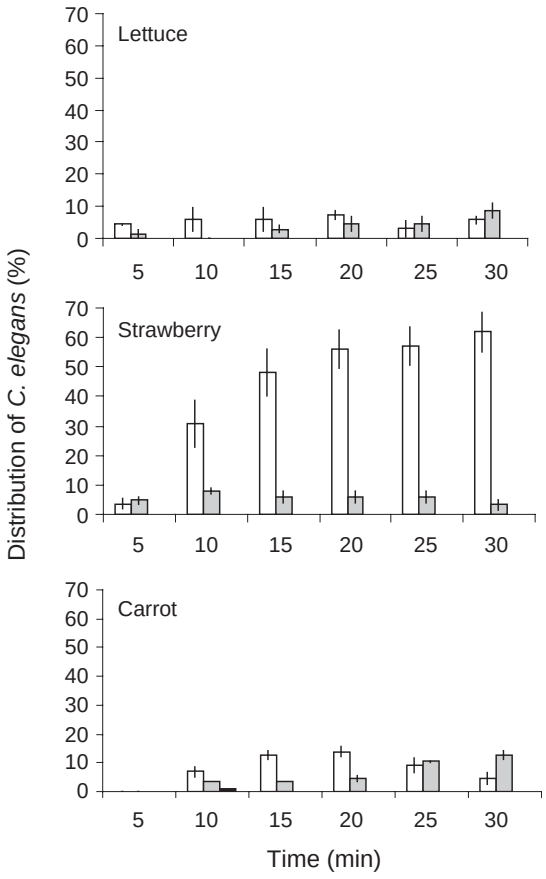

Fig. 2. Migration of *C. elegans* on TSAN on which lettuce, strawberry, carrot, bovine manure inoculated with *S. newport*, and uninoculated soil were deposited. Worms (20–30) were deposited 1.8 cm away from samples. The percentages of worms distributed in produce (open bars), manure (shaded bars), and uninoculated soil (solid bars) within 30 min at 20 °C were monitored. Some of the worms did not migrate to the produce, manure, or soil.

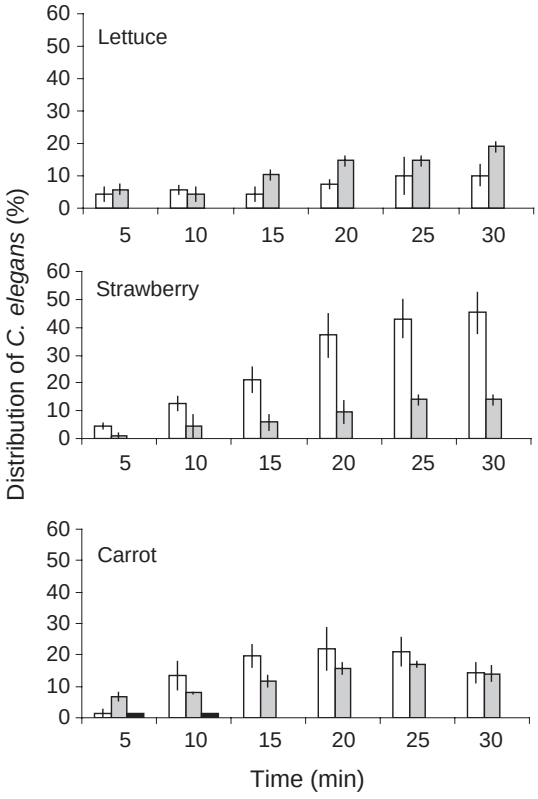

Fig. 3. Migration of *C. elegans* on TSAN on which lettuce, strawberry, carrot, bovine manure compost inoculated with *S. newport*, and uninoculated soil were deposited. Worms (20–30) were deposited 2.5 cm away from samples. The percentages of worms distributed in produce (open bars), bovine manure compost (shaded bars), and uninoculated soil (solid bars) within 30 min at 20 °C were monitored. Some of the worms did not migrate to produce, manure compost, or soil.

higher than the percentage migrating to lettuce. Moderate numbers of *C. elegans* migrated to carrots.

The percent distribution of *C. elegans* in produce, bovine manure compost, and soil is shown in Fig. 3. At all incubation times except 10 min, a higher percentage of *C. elegans* was located in bovine manure compost than was associated with lettuce. Higher numbers of *C. elegans* were observed in bovine manure compost (Fig. 3) compared to bovine manure (Fig. 2). Strawberries are highly aromatic and this may have contributed to the strong attraction of *C. elegans*. Worms moved more slowly toward carrot than lettuce or strawberries (Fig. 3). A large percentage of worms were not attracted to bovine manure compost or soil on plates also containing carrot.

Caldwell et al. (2003b) showed that *C. elegans* is more attracted to colonies of *S.* Poona on agar than to cantaloupe juice. *S.* Poona may have released compounds that preferentially attracted *C. elegans*. In our study, bovine manure and bovine manure compost inoculated with *S. newport*, uninoculated soil, and each type of produce were placed on the surface of agar not more than 5 min before depositing worms. This procedure reduced the time that compounds from the

Table 2
Presence of *Salmonella newport* in soil and on lettuce, strawberry, or carrot in contact with the surface of soil

Material inoculated with *S. newport*	Presence of *C. elegans* in soil	Number of samples positive for *S. newport*																			
		Lettuce[a]					Strawberry[a]					Carrot[a]					No produce[b]				
		1	3	5	7	10	1	3	5	7	10	1	3	5	7	10	1	3	5	7	10
Bovine manure	–	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0	0	0	0	0
	+	0/0	2/1	0/0	0/0	0/0	1/1	0/0	2/0	0/1	2/2	0/1	0/0	0/0	0/1	1/1	0	0	0	1	0
Bovine manure compost	–	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/0	0	0	0	0	0
	+	1/1	0/0	0/0	0/0	0/0	0/0	1/0	1/0	1/1	2/2	0/1	1/0	1/1	1/1	1/1	0	0	0	0	1

[a] Number of samples positive for *S. newport* out of four 2-g samples (four replicate experiments) of soil and out of four pieces of lettuce, strawberry, or carrot analyzed (soil/produce). Samples were incubated for 1, 3, 5, 7, and 10 days at 21 °C.

[b] Number of samples of soil from the top 1-cm layer positive for *S. newport* out of four 2-g samples (four replicate experiments).

samples entered the air within the covered Petri dishes or could be absorbed by the agar. Results clearly indicate that *C. elegans* migrates preferentially to strawberries compared to other test materials.

3.4. Migration of S. newport in soil to lettuce, strawberries, or carrots

The numbers of pieces of lettuce, strawberries, and carrots positive for the presence of *S. newport* after incubating for 0, 1, 3, 5, 7, and 10 days at 20 °C on the surface of soil under which bovine manure or bovine manure compost inoculated with *S. newport* had been placed are shown in Table 2. The presence of *C. elegans* in soil significantly ($P \leq 0.01$) contributes to the contamination of produce with the pathogen. *S. newport* was detected on the surface of lettuce, strawberry, and carrot within 3, 1, and 1 days, respectively, when *C. elegans* was present in soil layered on top of manure inoculated with the pathogen and within 1, 7, and 1 days when the worm was present in soil layered on top of manure compost inoculated with *S. newport*. With one exception (strawberries, 7 days, compost inoculated with *S. newport*), the pathogen was not detected on the produce over the 10-day study when *C. elegans* was not present in the soil.

When produce was not placed on top of the soil, *S. newport* was detected in the top 1 cm layer of only 2 of 80 samples analyzed over the 10-day incubation period. One soil sample tested positive for the pathogen after 7 days and 1 sample was positive after 10 days. *C. elegans* fed with *E. coli* O157:H7 prior to being added to turkey compost-amended and unamended soils has been reported to contaminate the soils within 4 days (Anderson et al., submitted for publication).

Results indicate that *C. elegans* transports *S. newport* initially in manure and compost for distances of 5 cm to produce in contact with the surface of soil. Attraction of *C. elegans* to strawberries is stronger than attraction to lettuce or carrot, which is in agreement with observations in attraction experiments using agar (Figs. 2 and 3). Preharvest fruits and vegetable that attract *C. elegans* would theoretically have a higher probability of becoming contaminated with salmonellae and perhaps other enteric pathogens that may be present in soil compared with produce having lower attractant properties. Whether free-living, bacterivorous nematodes act as vectors of enteric pathogens in field settings is not known but observations do indicate that *C. elegans* can serve as a vector of *Salmonella* to produce and reinforces the importance of sanitizing produce prior to consumption. Field studies to confirm observations in the laboratory are warranted.

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